Role of the kinase activation loop on protein kinase C θ activity and intracellular localisation

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Abstract Multiple protein kinase C (PKC) θ species, identified in an erythroleukaemia cell line, have been characterised in terms of their molecular properties and intracellular distribution. PKCθs localised in the detergent-soluble cell fraction have an M_r of 76 kDa (θ-76) and contain Thr^{538} or pThr^{538} in the kinase activation loop. In contrast, PKCθs localised in the Golgi complex have an M_r of 85 kDa (θ-85) and, although unphosphorylated at Thr^{538}, are catalytically active. Strikingly, only θ-76 species which are unphosphorylated at Thr^{538} can undergo autocatalytic conversion to θ-85. Moreover, a Thr^{538} → Ala PKCθ mutant is constitutively localised in the Golgi complex, confirming that changes in the phosphorylation state of this residue play a pivotal role in the overall control of catalytic properties and localisation of this kinase.

Key words: Golgi complex; Erythroleukemia cell; Protein kinase C θ phosphorylation; Thr^{538} → Ala protein kinase Cθ; Thr^{538} → Glu protein kinase Cθ

1. Introduction

Protein kinase C (PKC) θ is a member of the novel PKC isozyme subfamily which is activated by lipid cofactors [1]. The highest expression levels of this kinase have been detected in skeletal muscle and haematopoietic cells [2], but an increasing number of cell types, including endothelial, melanoma and mast cells, hepatocytes and nerve cell lines have also been shown to express PKCθ [3–8]. This kinase has distinct functions according to the cell type. In non-haematopoietic cells it is involved in the mechanisms underlying actin polymerisation and cell motility [3,9]. Conversely, in mature T cells PKCθ plays a non-redundant role in cell activation and proliferation processes [10]. The involvement of PKCθ in murine erythroleukaemia (MEL) cell cycle regulation has also been hypothesised, as this kinase is recruited to the mitotic spindle during cell division and is down-regulated in growth-arrested cells [11]. In any case, activation of PKCθ functions is preceded by its translocation from a soluble compartment to specific cell microdomains as lipid rafts, cytoskeletal structures or a supramolecular activation complex. These relocations of PKCθ involve interaction of this kinase isozyme with membrane lipids or with guanine nucleotide exchange factors; they also involve autophosphorylation as well as transphosphorylation events that are catalysed by protein kinases activated in different conditions [12–14]. Among the transphosphorylation sites, Thr^{538} in the activation loop of PKCθ has been indicated as a target for PDK1 activity, and the presence of pThr^{538} has been proposed as a critical requirement to produce a catalytically competent PKCθ [15,16]. However, although inactive on exogenous protein targets, PKCθ unphosphorylated at Thr^{538} has been found to be able to undergo autophosphorylation at Ser^{676}, a residue localised in the turn motif of the C-terminal region [16]. Here, we demonstrate that the phosphorylation state of Thr^{538} plays a crucial role in the control of both PKCθ catalytic properties and intracellular localisation.

2. Materials and methods

2.1. Cell culture and transfections

C44 MEL cells were obtained and cultured as specified previously [17]. Total RNA was obtained from MEL cells and reverse transcriptase-polymerase chain reaction was carried out as described [8]. The primers used to construct a pEF1/V5-His B (Invitrogen) expression vector, encoding PKCθ cloned in the *Not*I/*Xba*I sites, were based on the cDNA sequence of mouse PKCθ (GI 6679352). They were: sense 5′-AATGCGGCCGCATGGCACCGTTTCTTCG-3′; antisense 5′-AATTTCTAGACAGGAGCAAATGAGAGTC-3′. Cell transfections were carried out using 2×10^6 cells, 4 μg DNA and 8 μl DMRIE-C (Invitrogen) following the manufacturer's instructions. Recombinant PKCθ was immunoprecipitated from transiently transfected cells using an anti-V5 antibody (Invitrogen) and the procedure described previously [11]. T^{538} → A and T^{538} → E PKCθ mutants were generated using the QuickChange XL Site-Directed Mutagenesis kit from Stratagene, and were completely sequenced.

2.2. Confocal microscopy

Cells were fixed, permeabilised and stained in the conditions specified previously [11]. The antibody dilutions were: 1:50 for monoclonal PKCθ N-terminal antibody raised against the 10–214 amino acid residue fragment of human PKCθ (Transduction Laboratories), 1:100 for PKCθ C-terminal antibody (Santa Cruz Biotechnology); 1:200 for V5 antibody (Invitrogen). Secondary antibodies were: anti-mouse Ig Alexa Fluor 488 conjugate (Molecular Probes), 1:300 dilution; anti-mouse Ig (H+L) Texas red conjugate (Calbiochem), 1:100 dilu-

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Abbreviations: PKC, protein kinase C; MEL, murine erythroleukaemia; PMA, phorbol myristate acetate; MBP, myelin basic protein; WGA, wheat germ agglutinin; PP, PKCθ pseudosubstrate peptide

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tion; anti-rabbit Ig Alexa 488. Chromatin was stained by incubating fixed cells with 2 μg/ml propidium iodide (Sigma Aldrich) for 5 min. Wheat germ agglutinin (WGA) Alexa Fluor 488 conjugate (2 μg/ml) was used to visualise the Golgi complex.

2.3. Immunoprecipitation and assay of PKCθ activity

Cells were lysed and PKCθ was immunoprecipitated from 5×10^6 C44 MEL cells [11], using 2 μg of the PKCθ antibody specified elsewhere. Immunoprecipitates were suspended in 30 μl of 20 mM Tris, pH 7.4, 0.14 M NaCl, 2.5 mM EDTA, 2.5 mM EGTA, 15 mM $MgCl_2$, 1 mM sodium orthovanadate, 5 mM dithiothreitol, 1 μM ATP (Sigma Aldrich), 20 μCi [γ-^{32}P]ATP (Amersham Biosciences), and, where indicated, 3 ng phorbol myristate acetate (PMA; Sigma Aldrich) and 5 μg myelin basic protein (MBP; Sigma Aldrich). Reactions were carried out at 30°C and, at the times specified elsewhere, were stopped by adding sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) sample buffer and immediately heated at 100°C for 2 min. Where indicated, 2 μM PKCθ pseudosubstrate peptide (PP; Sigma Aldrich) was used as a PKCθ inhibitor.

2.4. Western blotting

Total cell samples, cell fractions or immunoprecipitates were subjected to SDS–PAGE (8% gel) and proteins were then transferred to nitrocellulose membranes (Bio-Rad) as specified previously [11]. Blocked membranes were incubated for 1 h with one of the following PKCθ antibodies: C-terminal 1:1000 dilution; monoclonal N-terminal 1:250 dilution; pThr^{538} 1:1000 dilution (Cell Signaling Technology); pSer^{676} 1:1000 dilution, gift from Dr S. Shaw and Dr Y. Liu [16]. V5 antibody was used at 1:5000 dilution. Immunoreactivities were revealed with peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology) developed with an enhanced chemiluminescence detection system (Amersham Biosciences) [11]. Quantification of immunoreactive bands was carried out with a dual-wavelength flying-spot scanner (Shimadzu Corporation).

2.5. Cell fractionation

Isolation of detergent-soluble and detergent-insoluble fractions was carried out as described [12] using 2×10^7 C44 MEL cells with the following modifications: cell lysing buffer contained 1% phosphatase inhibitor cocktail II (Sigma Aldrich) and 1% Nonidet P-40. After centrifugation at $200\,000\times g$ for 15 min at 4°C, the supernatant (detergent-soluble material) and the pellet (detergent-insoluble material) were collected, diluted in SDS–PAGE sample buffer and immediately heated at 100°C for 2 min.

3. Results

3.1. Identification of PKCθ species

In whole MEL cell lysates we identified two differently immunoreactive forms of PKCθ, using antibodies directed towards N-terminal and C-terminal regions of the kinase. As shown in Fig. 1A, both antibodies recognised a 76 kDa protein band, named θ-76, but the C-terminal antibody also detected an 85 kDa protein band, named θ-85. This immunoreactivity was eliminated by preincubation of the primary antibody with the immunising peptide, thus confirming the specificity of the isozyme immunorecognition. We examined the distribution of θ-76 and θ-85 in two cell fractions, consisting of detergent-soluble (cytosolic plus membrane fraction) and detergent-insoluble material. As shown in Fig. 1B, θ-76 was localised in the detergent-soluble material, whereas θ-85 was associated with the detergent-insoluble material. It has been shown that phosphorylation of PKC isozymes reduces their electrophoretic migration in SDS–PAGE [18]. To determine whether θ-76 and θ-85 are characterised by different phosphorylation patterns, we analysed their reactivity against two phosphopeptide antibodies directed towards pSer^{676}, a C-terminal autophosphorylation site, and towards pThr^{538}, a transphosphorylation site localised in the kinase activation loop. As shown in Fig. 1C, although both θ-76 and θ-85

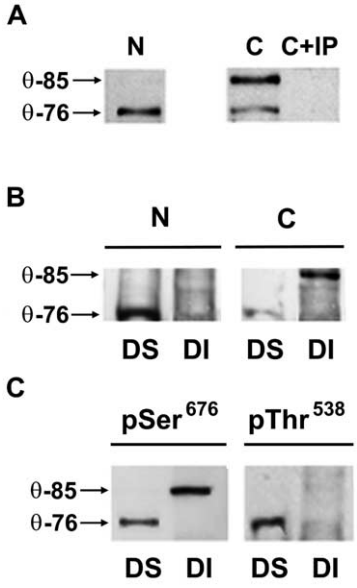

Fig. 1. Identification of PKCθ forms in MEL cells. Western blots were carried out with PKCθ N-terminal antibody (N), C-terminal (C) antibody or the specified phosphopeptide antibodies. A: 10^5 total cells. The reactivity of the C-terminal antibody preincubated with an excess of immunising peptide (IP) is also shown. B,C: Detergent-soluble (DS) and detergent-insoluble (DI) fractions from 10^5 cells. The above results were reproduced twice.

proved to be phosphorylated at Ser^{676}, only θ-76 was phosphorylated at Thr^{538}. Thus, unstimulated MEL cells contain distinct forms of PKCθ, distinguishable on the basis of their phosphorylation pattern and intracellular localisation.

3.2. In vitro conversion of θ-76 to θ-85 species

To determine whether θ-85, the slow-migrating PKCθ species in SDS–PAGE, can be produced by autophosphorylation of θ-76, the latter kinase form was immunoprecipitated from MEL cells using the PKCθ N-terminal antibody, which preferentially recognises θ-76 (see Fig. 1). Western blot analysis carried out with the PKCθ C-terminal antibody, which recognises both θ-76 and θ-85 species, revealed that the immunoprecipitated kinase consisted of a single immunoreactive band with an M_r of 76 kDa (Fig. 2A). Autophosphorylation of the isolated θ-76 form was then induced by adding PMA, as an activating lipid cofactor, in the presence of [γ^{32}P]ATP. After 5 min autophosphorylation, an additional immunoreactive band with the electrophoretic mobility of θ-85 was also detectable. The production of θ-85 was fully prevented by adding a PKCθ PP to the autophosphorylation mixture, confirming that the slow-migrating protein band was a true product of PKCθ autophosphorylation activity. We also analysed the immunoreactivity of the two PKCθ forms present at the end of the autophosphorylation assay by using the PKCθ N-terminal antibody. As shown in Fig. 2A, this antibody displayed a lower affinity for θ-85 than the C-terminal antibody did, thus explaining the discrepancy in the reactivity of these antibodies towards θ-76 and θ-85 reported in Fig. 1A. An autoradiography analysis was also carried out following the autophosphorylation reaction. As shown in Fig. 2B, both the θ-76 and θ-85 bands contained ^{32}P, indicating that autophosphorylation of the θ-76 species had not reached completion or, alternatively, that specific molecular constraints present in a

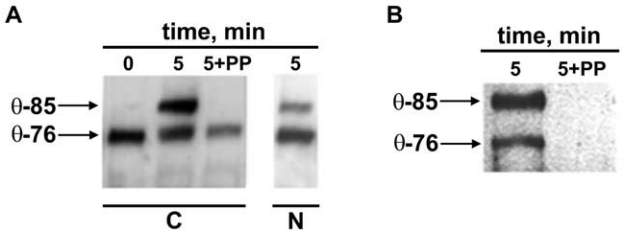

Fig. 2. Autophosphorylation of θ-76. θ-76 was immunoprecipitated from unstimulated MEL cells using PKCθ N-terminal antibody and subjected to autophosphorylation for 5 min in the absence or presence of 2 μM PKCθ PP. A: Western blot of the autophosphorylation products evaluated with PKCθ C-terminal (C) or N-terminal (N) antibody. B: Autoradiogram of corresponding lanes in A. Similar results were obtained in two additional experiments.

fraction of θ-76 molecules prevented the autophosphorylation steps required to produce their conversion to θ-85. We first analysed the extent of conversion of θ-76 to θ-85 at increasing times of autophosphorylation. As shown in Fig. 3, the reaction reached a steady state within 10 min, when approximately 60% of θ-76 was converted to θ-85. We excluded that this lack of completion in the conversion of the two forms was caused by inactivation of the kinase or consumption of ATP, as the enzyme was fully active on an exogenous substrate at any time of incubation. Furthermore, the kinetics of θ-76/θ-85 conversion was not affected by the presence of MBP in the incubation mixture, suggesting the occurrence of intramolecular autophosphorylation.

We then examined the phosphorylation state of Thr^{538} on both θ-76 and θ-85 throughout the autophosphorylation reaction. pThr^{538} was found to be stably confined to the θ-76 protein band, and any attempt to detect pThr^{538} on θ-85, by prolonging incubation times or adding phosphatase inhibitors, was unsuccessful (data not shown). Thus, we concluded that θ-76 molecules phosphorylated at Thr^{538} are not able to undergo conversion to θ-85 by autophosphorylation.

3.3. In vivo phosphorylation of PKCθ

To establish whether θ-76 and θ-85 are also subject to interconversion in vivo, cells were stimulated with PMA, and the detergent-soluble and detergent-insoluble cell fractions were analysed, at increasing times, by Western blot with different PKCθ antibodies. As shown in Fig. 4A, PMA induced a two-fold increase in the amount of θ-85 associated with the detergent-insoluble cell material, suggesting that PMA-promoted autophosphorylation of θ-76 caused its conversion to θ-85, which associated with the detergent-insoluble cell moiety. In this cell localisation, θ-85 persisted up to 60 min stimulation. However, a fraction of θ-76 did not undergo conversion and remained in the detergent-soluble fraction. To determine whether, in these conditions, θ-76 and θ-85 undergo changes in the phosphorylation state at Ser^{676} and Thr^{538} residues, the detergent-soluble and -insoluble fractions of unstimulated or PMA-stimulated MEL cells were subsequently analysed by Western blot with different phosphopeptide antibodies. As shown in Fig. 4B, pSer^{676} was detectable on both θ-76 and θ-85 at any time. When probed with the pThr^{538} antibody, the same blot showed that the level of phosphorylation on θ-76 in the detergent-soluble fraction remained almost constant throughout cell stimulation with PMA. In contrast, θ-85 in the detergent-insoluble fraction underwent phosphorylation at Thr^{538} following 15 min cell exposure to PMA. The slow kinetics of this phosphorylation step suggests that it is catalysed by a protein kinase which undergoes translocation and/or activation in these conditions.

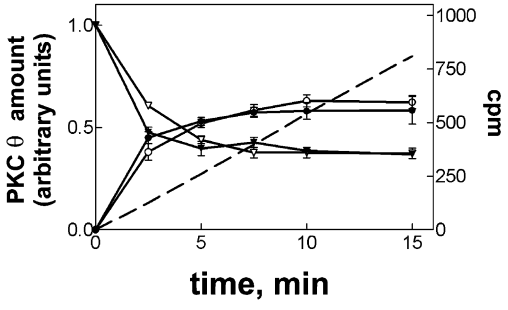

Fig. 3. Time course of θ-76/θ-85 conversion. θ-76 was immunoprecipitated as in Fig. 2 and its autophosphorylation was carried out in the absence (closed symbols) or presence (open symbols) of MBP. At the indicated times, the amounts of θ-76 (triangles) and θ-85 (circles) species were quantified by Western blot with PKCθ C-terminal antibody. Data are expressed as the mean ± S.D. of three separate experiments. ^{32}P incorporation in MBP was assayed as specified in Section 2 (dotted line).

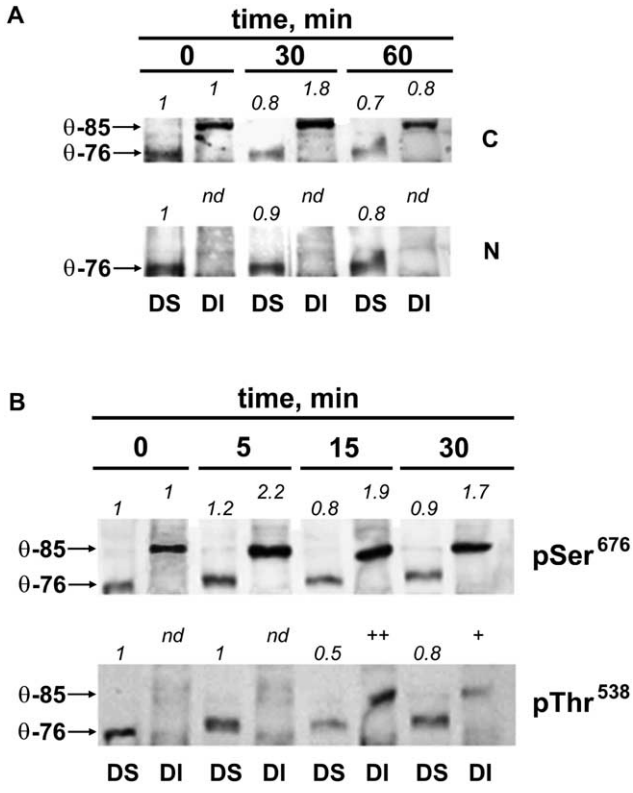

Fig. 4. Effect of PMA on θ-76 and θ-85 cell distribution and phosphorylation. A,B: Cells were stimulated with 100 ng/ml PMA for the indicated times, and detergent-soluble (DS) and detergent-insoluble (DI) fractions were analysed for θ-76 and θ-85 localisation with PKCθ C-terminal (C) or N-terminal (N) antibody. Phosphorylation at specific sites was evaluated with the indicated phosphopeptide antibodies. The numbers above lanes represent the intensity of the immunoreactive band calculated by densitometric scanning and assuming the arbitrary value 1 for the band present at 0 time in the detergent-soluble or the detergent-insoluble cell fraction, respectively. nd = not detectable. Similar results were obtained in two additional experiments.

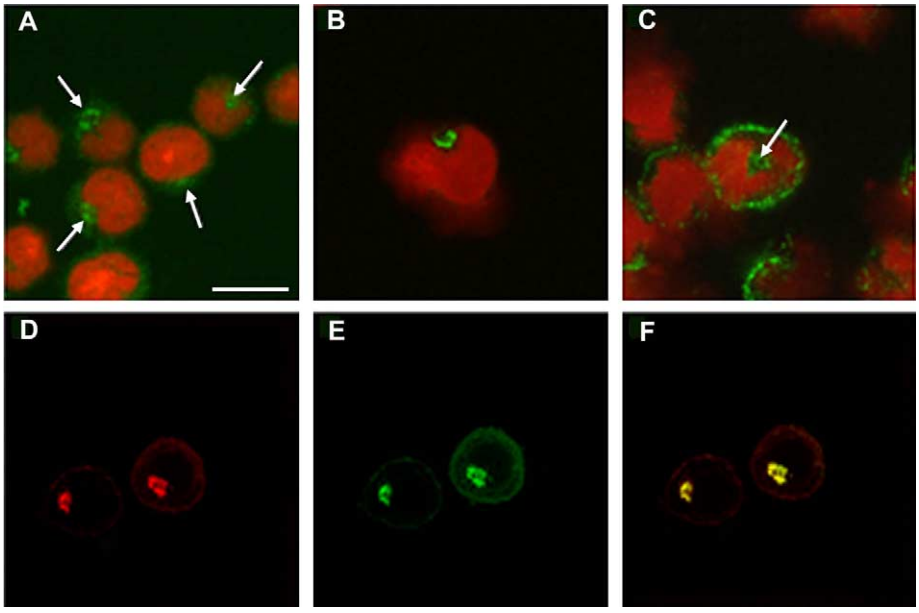

Fig. 5. Changes in intracellular distribution of PKCθ in PMA-stimulated cells. Wild-type (A–C) or $T^{538} \rightarrow A$ PKCθ transfected MEL cells (D–F) unstimulated (A,B,D–F), or stimulated for 5 min with PMA (C), were fixed and PKCθ localisation was evaluated using C-terminal PKCθ antibody (A–C) (green fluorescence) or anti-V5 antibody (D) (red fluorescence). The confocal microscopy image in B was acquired by photon counting mode. Chromatin was stained with propidium iodide (red fluorescence in A–C). WGA Alexa 488 was used to monitor the Golgi complex (green fluorescence in E). F: Merged image of D and E. Images are representative of data obtained on analysing at least 30 cells in random non-overlapping fields. Arrows indicate Golgi-associated PKCθ. Bar = 10 μm.

3.4. PKCθ intracellular localisations

To ascertain whether PKCθ recovered in the detergent-insoluble material is associated with specific cytoskeletal structures, the localisation of the kinase was analysed by confocal microscopy in unstimulated and PMA-stimulated cells. As shown in Fig. 5A,B, a fraction of PKCθ was detectable in the perinuclear cell region of unstimulated cells. Cells exposed to PMA showed a translocation of PKCθ to the plasma membrane (Fig. 5C), but, also in this condition, a fraction of PKCθ remained located on a discrete structure in the perinuclear region. Specifically, we identified a co-localisation between perinuclear PKCθ and Golgi structures (Fig. 5D–F).

To explore whether the intracellular localisation of PKCθ is controlled by the phosphorylation state of Thr^{538}, we produced MEL cells expressing $T^{538} \rightarrow A$ or $T^{538} \rightarrow E$ PKCθ mutants. $T^{538} \rightarrow A$ PKCθ showed an M_r of 88 kDa in Western blot (not shown) and an almost complete localisation in the Golgi area (Fig. 6). Strikingly, the intracellular distribution of this kinase mutant did not change in cells exposed to PMA. In contrast, $T^{538} \rightarrow E$ PKCθ showed an M_r of 79 kDa in Western blot (not shown), and a diffuse intracellular localisation (Fig. 6). Brief cell stimulation with PMA produced translocation of the kinase to the plasma membrane. These findings support the idea that a non-phosphorylatable residue, or a residue that mimics stable phosphorylation at the 538 position in the kinase activation loop, crucially modifies the ability of PKCθ to be localised in specific cell compartments.

3.5. Catalytic activity of PKCθ from MEL cells

The presence of θ-85 species associated with the Golgi complex, but devoid of $pThr^{538}$, raised the question of their catalytic activation. To determine whether θ-76 and θ-85 express catalytic activity on exogenous protein targets, both PKCθ species were isolated by immunoprecipitation from the detergent-soluble and detergent-insoluble fractions of MEL cells, respectively. The catalytic activity was then assayed on MBP as a substrate. As shown in Table 1, θ-76 activity was dependent on the presence of PMA in the assay mixture. In contrast, θ-85 was active in the absence of any added lipid cofactor, but inactive when PMA was added to the assay mixture. Recombinant $T^{538} \rightarrow A$ PKCθ, also isolated by immunoprecipitation, displayed a catalytic behaviour similar to θ-85. These

Table 1

PKCθ species	Kinase activity (cpm)		
	−PMA	+PMA	−PMA+PP
θ-76	180 ± 50	1800 ± 200	110 ± 35
θ-85	1500 ± 200	310 ± 60	160 ± 45
$T^{538} \rightarrow A$	1650 ± 180	200 ± 40	150 ± 60

θ-76 was immunoprecipitated from the detergent-soluble and θ-85 from the detergent-insoluble material of C44 cells. $T^{538} \rightarrow A$ PKCθ was isolated by immunoprecipitation with V5 antibody from cells 24 h after transfection. Aliquots of the immunoprecipitates were assayed for kinase activity as specified in Section 2. After 5 min at 30°C, reactions were stopped by adding SDS–PAGE sample buffer, and samples were immediately heated at 100°C for 2 min.
After electrophoresis MBP bands were identified by autoradiography and then cut; ^{32}P incorporation was measured in a Beckman LS6500 scintillation counter. Values are the mean ± S.D. of three separate experiments.

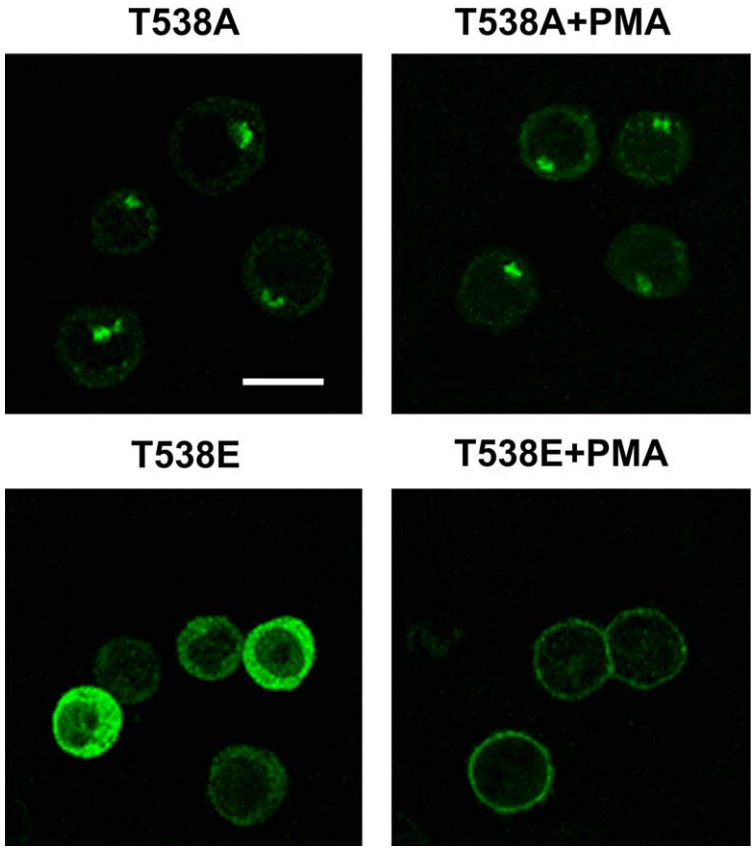

Fig. 6. Intracellular localisation of PKCθ mutants. Cells expressing the indicated PKCθ mutants were fixed 24 h after transfection and stained with V5 antibody followed by an Alexa 488-conjugated secondary antibody. Left panels represent unstimulated cells, right panels represent 1 min PMA-stimulated cells. Bar = 10 μm. The results are representative of data obtained on analysing at least 30 cells in random non-overlapping fields.

observations indicate that PKCθ species localised in the Golgi region, although lacking pThr^{538}, do not require exogenous activating cofactors to express catalytic activity towards protein targets.

4. Discussion

In this study, we identified different PKCθ species and analysed their molecular and catalytic properties. We recovered a PKCθ species associated with the detergent-insoluble fraction in both unstimulated and PMA-stimulated MEL cells. This kinase is easily distinguishable from PKCθs localised in the detergent-soluble cell fraction, as it shows a gel shift from an M_r of 76 to 85 kDa in SDS–PAGE. We demonstrated that in vitro the 76 kDa PKCθ (θ-76) can be converted by autophosphorylation to a new species with lower electrophoretic mobility and an apparent molecular mass of 85 kDa (θ-85). With regard to autophosphorylation sites, Ser^{676} residue in the turn motif of PKCθ proved to be constitutively phosphorylated, as both θ-76 and θ-85 were recognised by a pSer^{676}-specific antibody in unstimulated and PMA-stimulated cells. Thus, we excluded that autophosphorylation at this site is sufficient to promote the observed gel shift of θ-76 to θ-85. The hydrophobic motif peptide, which is conserved among PKC isozymes and has been proposed as a molecular switch in the partitioning of PKCs between detergent-soluble and -insoluble cell fractions [13], also proved to be constitutively phosphorylated at Ser^{695} on both θ-76 and θ-85 in MEL cells (M. Passalacqua, unpublished data). Although the phosphorylation of this peptide could participate in regulating the affinity of the various PKC isozymes for specialised intracellular docking sites in unstimulated MEL cells, the presence of pSer^{695} also in θ-76 suggests that θ-76/θ-85 conversion is not due to PKCθ autophosphorylation at this site. Putative nPKC substrate sequence motifs, characterised by the presence of basic residues between the −4 and −2 positions and a hydrophobic residue at the +1 position [19], are present at Ser^{370} and Ser^{433} in a region of PKCθ stretching from the end of the regulatory to the beginning of the catalytic domain. At present, we can only speculate that these favourable sites of autophosphorylation might participate in θ-76/θ-85 conversion by a *cis* or *trans* catalytic mechanism which in any case produces a species with reduced immunoreactivity to the PKCθ N-terminal antibody. Further experiments are necessary to identify these autophosphorylation sites.

Treatment of θ-85 with both alkaline phosphatase and protein phosphatase λ was not sufficient to obtain its re-conversion to θ-76 (data not shown). This result suggests that autophosphorylation of θ-76 leads to a phosphatase-resistant PKCθ form. The presence of a molecular constraint, which controls both accumulation of phosphate at other PKC sites and phosphatase sensitivity, has also been described previously for the PKCα isozyme [20].

Interestingly, the θ-76/θ-85 protein conversion is restricted to θ-76 molecules not phosphorylated at Thr^{538}. Hence, although the detergent-soluble θ-76 pool is composed of dif-

ferent kinase species that contain Thr^{538} or pThr^{538} in the kinase activation loop, only θ-76 kinases not phosphorylated at this site are able to catalyse the autophosphorylation steps that result in the conversion to θ-85. Surprisingly, also θ-85 species found to be associated with the detergent-insoluble fraction of unstimulated MEL cells do not contain any detectable pThr^{538}. The relevance of the Thr^{538} phosphorylation state in the intracellular distribution of PKCθ is supported by evidence obtained by using PKCθ mutated at this amino acid residue. Substitution of Thr^{538} with a non-phosphorylatable residue is sufficient to induce association of the kinase with the Golgi complex; conversely, its substitution with a residue that mimics pThr [16] promotes a preferential cell-soluble localisation.

We have shown that Golgi-associated θ-85 can be subsequently phosphorylated at Thr^{538} in cells stimulated with PMA. PDK1 has been proposed as a potential kinase involved in Thr^{538} phosphorylation [15], and a physical interaction between PKCθ and PDK1 has also been described [16]. In any case, the slow kinetics of Thr^{538} phosphorylation observed in PMA-treated MEL cells suggests that the protein kinase responsible for PKCθ activation loop phosphorylation either needs to be recruited to the detergent-insoluble compartment containing θ-85, or requires an activation process to recognise θ-85 as a substrate.

Thr^{538} phosphorylation has been shown to be a critical requirement for the catalytic competence of PKCθ. In agreement with previous observations [16], we found that T^{538}→A PKCθ is inactive when assayed in the presence of PMA. However, surprisingly, both wild-type θ-85 and T^{538}→A PKCθ Golgi-associated species were able to phosphorylate an exogenous substrate in the absence of PMA. Previous reports also described an inhibitory effect of lipid molecules on different PKC isozymes [21,22], but the molecular details of the inhibition of PKCθ species by PMA remain to be explored.

The presence of an active PKCθ associated with the Golgi complex can be ascribed to a co-immunoprecipitated activating cofactor. In mitotic cells the catalytic activation of a PKCθ species localised in centrosomes and kinetochores has been shown to depend on the interaction of this kinase with a protein cofactor [23]. To date, the activating cofactor of PKCθ species translocated to the Golgi complex in interphase cells has not been identified. PKCε and δ isozymes, which, like PKCθ, belong to the novel PKC subfamily, have been found to be transiently translocated to the Golgi complex [24,25]. However, the constitutive localisation of PKCθ species in this cell region, as well as the absence of its further translocation to the plasma membrane by PMA, suggests that a distinct molecular mechanism is involved in the process of PKCθ recruitment to the Golgi complex. This conclusion is further supported by recent evidence of a constitutive localisation of the PKCθ regulatory domain in the Golgi complex in Jurkat cells [26]. Divergent primary structures, shown specifically in the regulatory domains of the homologous PKCδ and θ isozymes, may be responsible for their different interaction with Golgi structures.

Studies are in progress to explore this hypothesis and to define the functional role of the different PKCθ species in MEL cells.

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